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Advantages of a miniature pig model in research on human hereditary hearing loss

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Abstract

In medical laboratory animals, the pig is the closest species to human in evolution, except for primates. As an animal model, the pig is highly concerned by many scientists, including comparative biology, developmental biology, medical genetics. Rodents as animal model for human hearing defects has are poor producibility and reliability, due to differences in anatomical structure, evolutionary rate and metabolic rate, but these happens to be the advantages of the pig model. In this paper, we will summarize the application of miniature pig in the study of human hereditary deafness.

Keywords: Mini pig; Animal model; Hereditary deafness

1. Deafness is ranked the second among disabling diseases in China. More than half of congenital deafness are hereditary hearing loss induced by a variety of causal genetic mutations via complex pathogenic mechanisms. It is of great significance to advance basic research on hereditary deafness and its translation to the clinic

Deafness is one of the top two most common diseases that significantly impact quality of life in humans. According to the WHO Fact sheet No 300 "Deafness and hearing loss" (http://www.who.int/mediacentre/factsheets/fs300/en/), over 5% of the population in the world, about 360 million people, suffer from disabling hearing loss (328 million adults and 3.2 million children). Prevalence data indicate that the rate of children with congenital deafness in different countries is 1/1000 to 3/1000. In China, deafness is the second leading disabling disease, and China is also the country with the largest number of patients with hearing disorders. According to the second survey on disabled persons in China in 2006, there are 27.8 million people with hearing disability in China and the number is believed to increase by 30,000 every year, half of which caused by hereditary hearing loss.

Hearing impairment induced by genetic factors mainly result from gene mutations that impair the gene or regulatory elements responsible for ear development, structural integrity and functioning. Currently known deafness inheritance patterns include autosomal recessive (DFNB), autosomal dominant (DFNA), X-linked (DFN) and mitochondrial inheritances. Up-to-date, more than 137 causal genes have been identified in syndromic and nonsyndromic hearing loss. Products of these genes are part of cytoskeleton structures, cellular junctions, membrane transporter proteins, ionophorous proteins, regulatory elements and other extracellular matrix and structural components in the inner ear. The number of estimated hearing related genes is in the range of 250–300, although many of these genes lack clear descriptions. For some genes, little is

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known regarding their functions and involvement in pathological conditions. Currently, deafness gene screening in China is limited to mutations of the GJB2, SLC26A4, mtDNA (A1555G and C1494T) genes. Genetic deafness carries a high degree of genetic heterogeneity, i.e. one hearing loss phenotype can be caused by mutations in different genes, while it is also possible for different point mutations in the same deafness gene to result in different phenotypes or genetic patterns. This increases the complexity of studying gene functions and their roles in pathogenesis.

Furthermore, most known genetic mutations can cause changes of inner ear structures and lead to sensory hearing loss, with few of them also causing middle ear deformities and conduction deafness from defects of the external or middle ear. Sensory hearing loss is a result of impairment of hearing pathway from the cochlea to auditory cortex. In some cases, conduction deafness is mild and easily treatable. While sensory hearing loss can be improved by cochlear implant or hearing aids, it often cannot be completely resolved. It is difficult to treat hereditary hearing loss, because most times it is sensory hearing loss. There is an urgent clinical demand to increase research efforts on hereditary hearing loss for an effective solution.

2. Animal models have an irreplaceable role in discovering deafness genes, verifying their functions, studying genetic deafness pathogenesis, and evaluating related diagnosis, prevention and treatments. Reported animal models of genetic deafness are mainly based on mouse and other rodents, whose cochlear size, inner ear development and gene functions are very different from people

In vivo studies of human hereditary deafness are complicated by phenotypic differences, complex genetic background, small sample sizes and difficulties in obtaining research materials (inner ear tissue, etc.). Animal models therefore have irreplaceable role in genetic deafness studies. The rapidly development of genomics has greatly improved the efficiency of cloning and identification of heredity deafness genes. Today, more than 63 heredity deafness genes have been detected via genetic analysis of deaf families (Friedman et al., 2007). Discovery and cloning of these genes are useful for us to understand hearing mechanisms, improve diagnosis of hearing disorders, develop relevant treatments and conduct prenatal genetic screening, among other benefits (Dror and Avraham, 2009). There are an estimated 250–300hearing related genes, although many of them are yet to be clearly described, let alone understanding their functions or their involvement in pathologies. Although human studies directly reflect conditions in humans, limitations include the following: 1) Genetic linkage analysis requires large genetic families with large numbers of family members. Genetic analysis of sporadic occurrence of hereditary deafness among small numbers of family members is very difficult. 2) Mating between individual deafness patients from different families results in hereditary deafness families carrying more than 2 mutations, which make analyzing the genetic linkage in these families difficult. 3) Research on hereditary hearing loss goes beyond discovering and cloning deafness genes. Prevention and treatment of hereditary deafness require in-depth understanding of gene functions and their roles in the hearing system, which is difficult to be achieved in human in vivo studies, as such studies are restricted to postmortem sampling in deaf patients, yielding extremely limited study materials. In contrast, sampling is possible at any stage of development of any deafness disease in animal models, including the embryonic development period to explore interaction between genes and proteins, as well as their temporal and spatial expression and biological characteristics. In addition, deafness animal models serve as an irreplaceable tool widely used in cochlear implantation, stem cell therapy and gene therapy, and more. Using animal models, it is possible for researchers to understand the inner ear pathway mechanisms under normal and pathological conditions to ultimately develop cell and gene therapies that can be used in humans.

Today, more than 400 animal models of genetic deafness have been reported, including genetic engineering animal models, EUN chemical mutagenesis animal models and spontaneous mutation animal models. Genetic engineering animals are homologous gene defect animal models carrying human deafness related defect genes and are derived from gene targeting or knockout. It makes the profound study about pathogenic mechanisms of defect genespossible. EUN chemical mutagenesis and spontaneous mutation animal models are useful in finding novel deafness pathogenic genes in animals and equivalent genes in humans. Genetic deafness animal models relate to protein encoding genes, tRNA or rRNA genes and microRNA, etc (Guo et al., 2015a,b), encoding transcription factors, ion channels, transporter proteins, extracellular matrix, gap junction proteins, adhesion proteins, myosin, cytoskeletal proteins and microRNAs that regulate target gene expression.

However, at present animal models used in hereditary hearing loss research use mainly mice and other rodents, with certain inherent biological characteristics limiting further investigation in many areas. There are significant differences in embryonic development between human and rodents. Some human developmental disorders cannot be reproduced in a mouse model (Smithies, 1993). Developmental differences between human and mice involve the inner ear. Mice and rodents have a late maturing auditory system with no hearing at birth. Their inner ear continues to develop until 14 days after birth and only then can these animals acquire hearing ability. Breakthroughs are urgently needed in studies on hereditary hearing loss, hair cell regeneration, artificial hearing reconstruction and early hearing rescue, but such studies cannot be carried out in mouse models. Although rodents play an important role in the discovery and research of deafness genes, most deafness genes derived from mouse models have failed to be clearly linked with phenotypes of human deafness (Friedman et al., 2007). In addition, phenotypes of deafness gene mutations are different between humans and mice. For instance, genetic knock-in mice with mutated SLC26A4 gene (p.H723R),

commonly foundamong East Asia populations, are normal in ether hearing or vestibular functions with no pathological morphology changes in the inner ear. It is suggested that there are significant differences in the development of auditory system and in mechanisms of disease between humans and mice.

In studying pathogenic mechanisms and prevention and treatment of hereditary hearing loss, there is a shortage of models of large size animal sharing similarities to humans. Creating such a model with similar genetic and disease characteristics to humans that can be used for surgical training and provides easy translation of study results to clinical applications is of important values.

3. Pig deafness models share more similarities to humans in both genetic and disease characteristics than rodent models, and can be used for surgical training. Translation of pig study results to clinical applications is also easier. Pig models, as large animal models, carry unique advantages in research on pathogenic mechanisms of hereditary deafness, in developing clinical treatments and in evaluating medical devices

Second to only primates, pigs are aspecies closest to humans and have been used in medical research. The auditory organ in pigs and humans share high levels of similarity in structure. Studies have shown that the middle ear and pharyngeal lymphoid tissue in pigs are very similar with those in humans. Pigs are very suitable for models of otitis media (Pracy et al., 1998). The porcine cochlea has been widely used in developing surgical techniques for cochlear fenestration (Coulson et al., 2008) and cochlear fine tomography (Sepehr et al., 2008), as the thickness and density of its bony wall is similar to those of people. Our previous studies have found that the size and morphology of pig cochlea are very similar to that of humans. Also similar with humans, the inner ear is essentially fully developed in pigs at birth with normal hearing ability (Guo et al., 2015a,b). Compared with rodents which have delayed maturation of their auditory system and require continued inner ear development after birth before gaining normal hearing, the pig, as a large animal model, demonstrates promising prospect of applications in the field of otological research.

Our group has developed several porcine gene mutation models of deafness. In the first model, we identified a mutation in a non-regulatory region of melanocyte-specific promoter of microphthalmia-associated transcription factor gene (Mitf), which can cause early degeneration of intermediate cells in cochlear SV and profound hearing loss, the typical phenotype of Waardenburg syndrome in humans.

Another pig model of congenital deafness that we have developed is created by chemical mutagenesis using N-ethyl-N-nitrosourea (ENU) exposure in male pigs in order to generate mutant spam. A dominant mutant has been identified and characterized by white coat color and hearing loss. Histology data showed hypoplastic inner ear malformation in the mutant pigs and the causative mutation was mapped to the *Sox10* locus as a single amino acid change. This is an extremely useful model to further study the mechanism of genetic mutation causing Mondini dysplasia. Our data have demonstrated the advantages and efficiency of large-scale, phenotype-driven mutagenesis by ENU in pigs.

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